Imidacloprid as a potential agent for the systemic control of sand flies

Gideon Wasserberg[1,4✉], Richard Poché[2], David Miller[2], Michelle Chenault[3], Gabriela Zollner[1],
and Edgar D. Rowton[1]

[1]*Division of Entomology, Walter Reed Army Institute of Research, 530 Robert Grant Ave., Silver Spring, MD 20910, U.S.A.*
[2]*Genesis Laboratories, P.O. Box 1195, Wellington, CO 80549, U.S.A.*
[3]*Food and Drug Administration, 10903 New Hampshire Avenue, White Oak 66, Silver Spring, MD 20993, U.S.A.*

ABSTRACT: Our goal was to study the effectiveness of the insecticide imidacloprid as a systemic control agent. First, to evaluate the blood-feeding effect, we fed adult female *Phlebotomus papatasi* with imidacloprid-treated rabbit blood and monitored blood-feeding success and survival. Second, to evaluate the feed-through effectiveness of this insecticide, we fed laboratory rats and sand rats with insecticide-treated food and evaluated the survival of sand fly larvae feeding on rodents' feces. In the blood-feeding experiment, 89.8% mortality was observed with the higher dose (5 mg/ml) and 81.3% with the lower dose (1 mg/ml). In the larvicide experiments, both sand fly species demonstrated a typical dose-response curve with the strongest lethal effect for the 250 ppm samples. *Lutzomyia longipalpis* larvae, however, were less sensitive. In all experiments, 1st instar larvae were more sensitive than the older stages. First instar *P. papatasi* larvae feeding on sand rat feces passed the larvicidal threshold of 90% mortality at doses higher than 50 ppm. In comparison, in older stages 90% mortality was obtained with a dose of only 250 ppm. Overall, results support the feasibility of imidacloprid as a systemic control agent that takes advantage of the tight ecological association between the reservoir host and the sand fly vector. ***Journal of Vector Ecology*** **36 (Supplement 1): S148-S156. 2011.**

Keyword Index: Systemic control, sand fly larva control, imidacloprid, sand rat, feed-through.

INTRODUCTION

Systemic control of arthropod pests and plant disease vectors is quite common in agricultural systems (Isman et al. 1991, Kloepper et al. 2004, Weintraub 2007, Grafton-Cardwell et al. 2008, Bruck et al. 2009). In such systems, an insecticide is taken up by the roots or absorbed through the leaves into the plant, thereby providing long-term protection for the whole plant and assisting in reducing the pest's population while minimizing the environmental impact. In contrast, systemic control of human or animal disease arthropod vectors is unfortunately fairly underdeveloped (Service 2008, Mullen and Durden 2009). Systemic control has a particularly strong potential for vector-host associations characterized by a high degree of ecological coupling where the vector has a relatively narrow range of hosts (high intimacy) and depends on its host not only as a source of blood meals but also as a provider of habitat and shelter. Hence, this approach is mainly used with the control of ectoparasitic vectors such as fleas, ticks and mites where the host also constitutes the habitat (Kuris et al. 1980). Systemic control in these systems has been applied as part of an effort to control sylvatic plague and Lyme disease and usually involves oral delivery of insecticide-treated baits (Davis 1999, Slowik et al. 2001, Metzger and Rust 2002, Borchert and Poché 2006, Borchert et al. 2009). For example, Slowik et al. (2001) used orally-delivered bait treated with the development inhibitor fluazaron to control woodrat fleas but was unable to control ixodid ticks. Borchert et al. (2009) used bait containing imidacloprid to dramatically reduce levels and prevalence of flea infestations in the California ground squirrel. Further work in this field was performed by this group using an application of a range of orally-delivered insecticides (fipronil, imidacloprid, cythioate, nitenpyram, and selamectin) (Borchert and Poché 2003, Borchert and Poché 2004, Borchert and Poché 2006, Borchert et al. 2009).

Sand flies (Diptera: Psychodidae) are the vectors of leishmaniases worldwide (Killick-Kendrick 1999, Ashford 2000). Their degree of ecological coupling with their hosts ranges from loosely coupled systems (e.g., most vectors of visceral leishmaniasis) to tightly coupled associations such as that of *Phlebotomus papatasi* (Scopoli) and *Psammomys obsesus* (Cretzchmar) (Ashford 1996). *Phlebotomus papatasi* is the vector and *P. obesus* is one of the main reservoir hosts of *Leishmania major* (Yakimoff and Schokhor) (Kinetoplastida: Trypanosomatidae), which is the etiologic agent of cutaneous leishmaniasis in arid regions of North Africa and the Middle East (Ashford 1996, Saliba and Oumeish 1999). *Psammomys obesus* (180 g) is a diurnal, herbivorous rodent that feeds mostly on halophytic Chenopodiaceae plants (Daly and Daly 1973). It is gregarious and usually inhabits complex burrow systems that are often located underneath the host plant. Within the burrow, organic matter, in the form of plant debris and feces tends to accumulate. This, together with the relatively warm and moist ambient microclimate, provides ideal breeding conditions for *P. papatasi* larvae and diurnal

---

[4]Current address: Department of Biology, University of North Carolina at Greensboro, Greensboro, NC 27402, U.S.A.

Report Documentation Page	*Form Approved* *OMB No. 0704-0188*

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE **MAR 2011**	2. REPORT TYPE	3. DATES COVERED **00-00-2011 to 00-00-2011**

4. TITLE AND SUBTITLE **Imidacloprid As A Potential Agent For The Systemic Control Of Sand Flies**	5a. CONTRACT NUMBER 5b. GRANT NUMBER 5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER 5e. TASK NUMBER 5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Walter Reed Army Institute of Research,530 Robert Grant Ave,Silver Spring,MD,20910**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR’S ACRONYM(S) 11. SPONSOR/MONITOR’S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

Journal of Vector Ecology, March 2011

14. ABSTRACT

Our goal was to study the effectiveness of the insecticide imidacloprid as a systemic control agent. First, to evaluate the blood-feeding effect, we fed adult female Phlebotomus papatasi with imidacloprid-treated rabbit blood and monitored blood-feeding success and survival. Second, to evaluate the feed-through effectiveness of this insecticide, we fed laboratory rats and sand rats with insecticide-treated food and evaluated the survival of sand fly larvae feeding on rodents?feces. In the blood-feeding experiment, 89.8% mortality was observed with the higher dose (5 mg/ml) and 81.3% with the lower dose (1 mg/ml). In the larvicide experiments, both sand fly species demonstrated a typical dose-response curve with the strongest lethal effect for the 250 ppm samples. Lutzomyia longipalpis larvae, however, were less sensitive. In all experiments, 1st instar larvae were more sensitive than the older stages. First instar P. papatasi larvae feeding on sand rat feces passed the larvicidal threshold of 90% mortality at doses higher than 50 ppm. In comparison, in older stages 90% mortality was obtained with a dose of only 250 ppm. Overall, results support the feasibility of imidacloprid as a systemic control agent that takes advantage of the tight ecological association between the reservoir host and the sand fly vector.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**9**	

refuge for the adults, as well as an easy blood meal source for the females (Ashford 1996). Hence, from all aspects, this natural transmission cycle of *L. major* is highly focal and revolves around the fat sand rat and therefore constitutes a potential model system for testing the systemic control approach in flying hematophagous insects.

Control of sand flies comprises three general approaches: personal protection, reservoir host control, and sand fly control with most effort devoted to the latter using residual spraying of insecticides (Ashford 1999, Alexander and Maroli 2003). This method exhibits varying degrees of success and appears to be more effective in urban than in rural areas (Alexander and Maroli 2003). Systemic control of adult sand flies feeding on dogs wearing insecticide-impregnated collars was demonstrated for visceral leishmaniasis due to *L. infantum* (Killick-Kendrick et al. 1997, Maroli et al. 2001, Reithinger et al. 2004). Similarly, topical lotions, insecticidal baths, or shampoo treatments to dogs had a measurable success in reducing the transmission of visceral leishmaniasis due to *L. donovani* (Alexander and Maroli 2003). However, these approaches are impractical for *Leishmania* spp. with wildlife reservoirs. Therefore, systemic control using oral delivery of insecticide-treated baits holds a great promise for the control of leishmaniasis transmission in zoonotic systems characterized by a high degree of ecological coupling. Some work in this field has been already done. Kassem et al. (2001) showed that survival of adult female sand flies feeding on blood treated with avermectins was substantially reduced. Mascari et al. (2007a, 2007b, 2008) demonstrated the feed-through effectiveness of several insecticides (diflubenzuron, novaluron, and ivermectin) in reducing the survival of sand fly larvae feeding on feces of insecticide-fed hamsters.

The specific goal of our study was to provide a proof-of-concept for the effectiveness of imidacloprid as a systemic control agent of the sand fly vector of *L. major* by evaluating its effect on the survival of blood-feeding adult females and on coprophagic (feces-eating) larvae. Our hypothesis was that by systemically affecting the reservoir host, we could simultaneously reduce the survival of adult females and larval sand flies. To evaluate the broader use of this method, we used both a non-reservoir rodent model (*Rattus norvegicus* Berkenhout) and a reservoir host rodent (*P. obesus*) and compared the mortality responses between two larval developmental stages (1st and 2nd/3rd) for one native species and one non-native sand fly species (*P. papatasi* and *Lutzomyia longipalpis* Lutz & Neiva, respectively).

MATERIALS AND METHODS

Imidacloprid was used due to previous success of this product in other vector-rodent host systems (Borchert et al. 2009), its availability (i.e., it is not under patent), and low cost. Imidacloprid is a chloronicotinyl nitroguanidine insecticidal agent that acts on nicotinic acetylcholine receptors on the postsynaptic membrane, thereby causing insect central nervous system (CNS) impairment and death (Mullins 1993).

Of six laboratory rats (*R. norvegicus*) (three males and three females), housed individually (12 light:12 dark, 20-25° C, 30-70% RH), four rats were randomly chosen (two males and two females) and fed on imidacloprid diet for four days. The other two rats were fed a standard control diet which consisted of nutritionally complete Purina laboratory rodent pellets. Treatment diets were reformulated pellets incorporating an orange dye and 100 ppm imidacloprid for five days, followed by an eight-day washout period, followed by provision of pellets incorporating a green dye and 250 ppm imidacloprid for an additional five days. Water was provided *ad libitum*. Feces were collected and weighed daily and kept refrigerated in Ziplock bags labeled with the animal's cage identification number, study number, dosage level, and date. Feces from days 2 and 4 were collected for sand fly bioassays.

Twenty-two sand rats comprising six males and nine females for the treatment groups and three males and four females for the control (all assigned randomly) were used in this study. Sand rats originated from a colony at the Hebrew University, Israel, and were maintained under standard sand rat housing protocols developed by Dr. V. Michelle Chenault. Experimental diet was provided for seven days and consisted of a standard, nutritionally complete sand rat chow (Purina 5L09 test diet) that was reformulated with wax. Four different treatment diets were developed containing 0, 50, 100, and 250 ppm imidacloprid. The sand rat diets and water were provided *ad libitum*. All animals were monitored daily for general health and observations were recorded. Fecal samples were shipped without indication of treatment assignment for sand fly bioassays.

Samples of each imidacloprid-treated bait were evaluated by High Performance Liquid Chromatography (HPLC) at Genesis Laboratories, Inc. for verification of imidacloprid levels. Samples were prepared by grinding in a UDY mill, followed by methanol extraction. The supernatants were decanted, and the extraction procedure was repeated two additional times with fresh aliquots of methanol. An aliquot of each sample was filtered through a 0.20 μm syringe filter into a HPLC vial for analysis in comparison with prepared standards. Feces were collected from the Alpha-Dri bedding for the last five days of the seven-day treatment period. Feces were weighed and transferred to a plastic bag labeled with the animal's cage identification number and stored frozen at -20° C.

Sand fly bioassay

Phlebotomus papatasi originating from specimens collected in Israel (PPIS) and *Lu. longipalpis* originating from specimens collected in Jacobina, Brazil (LLJB), were from long-established colonies maintained by the Division of Entomology at Walter Reed Army Institute of Research (Silver Spring, MD). Immature sand flies were reared using a standard larval diet comprised of equal parts by volume of rabbit chow (5321 Rabbit Diet, LabDiet, PMI Nutrition International) and rabbit feces (Young et al. 1981) with a 1:4 volume of water which was composted in a Nature Mill Automatic Composter®.

Using the lab rat fecal pellets, we compared the survival of the two sand fly species (PPIS and LLJB) and two developmental stages (1st and 2nd/3rd instar). Bioassays comprised three levels of imidacloprid (0, 100, 250 ppm) and fecal pellets from two collection days (day two and four). Each dose-and-day treatment was replicated four times per bioassay and repeated twice. The control group was provided a standard larval diet (n = 6). Using sand rat fecal pellets, bioassays were performed with 1st and 2nd/3rd instars of PPIS. Bioassays comprised four levels of imidacloprid (0, 50, 100, 250 ppm) with five replicates per dose and a control group (n = 8). These bioassays were repeated three times.

Bioassays were conducted in 6-well culture plates (Corning Inc, Corning, NY) with 5 ml of plaster of Paris in the bottom of each well. The plaster was saturated with distilled water before the experiment and then blotted with filter paper immediately before use to remove any standing water.

Approximately 50 sand fly eggs were added to each well, allowed to hatch, and fed on regular diet for two to three days, after which ca. 0.1 g of crushed fecal pellets or regular larval feed (control) was added. Second-3rd instar larvae were obtained by allowing 1st instar larvae to grow on a standard diet in the wells until the larvae molted. At this time crushed fecal pellets or control feed (0.1 g) was added. Due to variation in hatching and growth time, this resulted in a mix of 2nd and 3rd instar larvae at a ratio of approximately 1:1. The wells of the plate were covered with parafilm to prevent larvae from escaping, and several holes were punctured with a needle to allow for ventilation. The wells were kept in a humidified room (26° C/75% RH) inside a container with a sponge saturated with water. The container was placed in an environmental chamber at 28° C, 90% RH, and a photoperiod of 14:10 (L:D). Larval mortality was recorded daily for seven days. Larvae were considered dead if they did not respond within 15 s to prodding with a blunt probe. Alimentation was noted by observation of the presence of frass in the vials and dark material in the guts of the larvae. All larvae were observed for abnormal behavioral and morphological characteristics.

Blood feeding bioassay

We used female PPIS (>46 sand flies per treatment) that were blood-starved for five days and sugar-starved for one day. We allowed them to imbibe through a chicken skin membrane (Tesh and Modi 1984) on defibrinated rabbit blood treated with two concentrations of imidacloprid (5 and 1 mg/ml) or the controls. Imidacloprid was dissolved in ethyl alcohol. We also used two controls: blood treated with 95% alcohol and untreated blood. Sand flies were allowed to feed for two hours. Then, live sand flies for each treatment were released into separate cages and the number of live engorged females was recorded. The number of dead engorged and non-engorged females was also recorded.

Statistical analysis

In the rat experiments, different treatments were applied to the same subject and pellet samples were taken from the same individual subject over time. In addition, larvae survival assays were based on repeated measures of the number of larvae alive over seven days. In the sand rat experiment, each individual was subjected to a single treatment; however, in the corresponding sand fly bioassays we replicated the effect of each treatment three times. To account for within subject correlation, that the response variable is count, and to control for differences in number of larvae at the beginning (N0) of the experiment, we used a random-intercept Poisson regression model with N0 as an offset (Rabe-Hesketh and Skrondal 2008) using STATA 10 (StataCorp. 2007). Risk-ratio was calculated as the inverse-log of the Poisson-regression coefficients (Rabe-Hesketh and Skrondal 2008). In the blood-feeding experiment, the difference in the proportion of surviving or blood-fed females was analyzed using Pearson's Chi-squared test with Yates' continuity correction.

RESULTS

Rat fecal pellets experiment

The survival of *P. papatasi* at the final day (day 7) of the experiment was approximately half of the survival of *Lu. longipalpis* (risk ratio = 0.55, p<0.0001). Therefore, we analyzed the results for each species separately.

P. papatasi. The significant negative interaction of dose-by-time (Table 1) indicates that the survival of *P. papatasi* larvae decreased faster with an increase in imidacloprid dose (Figure 1). This is true for both larval stages. However, survival of the later stages (2nd/3rd instar) was on average 51% higher than that of 1st instar larvae (Table 1). In addition, survival rate of larvae feeding on feces collected at day 4 of rat feeding was approximately 10% lower compared with those feeding on feces collected at day two (Table 1). If larvicide activity is defined as 90% mortality at the final day (day seven), all treatments showed a larvicide activity for 1st instar larvae except for 100 ppm day two pellets (Figure 1). In contrast, only the 250 ppm dose treatments showed larvicide activity for the 2nd/3rd instar. Survival rate of larvae fed on conventional feed vs those fed feces of untreated rats did not differ significantly (coef. = -0.014, se = 0.029, z=-0.48, P=0.629) (Figure 1).

Lu. longipalpis. As mentioned above, the survival of LLJB larvae feeding on feces of insecticide-treated rats was significantly higher than that of PPIS larvae. As with PPIS, survival of LLJB larvae decreased more rapidly at higher Imidacloprid doses (Table 2, Figure 2). Similarly, survival of 2nd/3rd instar larvae was significantly higher (approximately 34%) than that of 1st instar larvae (Table 2, Figure 2). Survival rate of larvae feeding on feces collected at day 4 of rat feeding was approximately 8% lower compared with those feeding on feces collected at day 2 (Table 2, Figure 2). However, neither of the treatments showed a 90% larvicidal activity with lowest survival (35.4%) occurring for 1st instar larvae feeding on day 4 fecal pellets of rats treated with a diet of 250 ppm imidacloprid (Figure 2). Similar to PPIS, larval survival did not differ between the two control groups (coef. = -0.0227, se = 0.0231, z=-0.99, P=0.324).

Table 1. Random-intercept poisson regression model of the effect of time, larval developmental stage, imidacloprid dose, and rat feces collection day (Day) on the number of surviving *P. papatasi* larvae feeding on lab rat feces. Statistical overdispersion is depicted by the parameter 'alpha'.

	Coef.	Std. Err	z	P	95% Conf. Interval	
Time	-0.0084	0.0067	-1.25	0.210	-0.0216	0.0047
Stage	0.4114	0.0244	16.80	0.000	0.3634	0.4594
Day	-0.1001	0.0115	-8.66	0.000	-0.1227	-0.0774
Dose	0.0002	0.0003	0.73	0.466	-0.0004	0.0009
Dose × time	-0.0019	0.0001	-22.30	0.000	-0.0021	-0.0017
Intercept	-0.6296	0.1411	-4.46	0.000	-0.9061	-0.3531
linit	(offset)					
lnalpha	-2.3236	0.5833			-3.467	-1.1803
alpha	0.097919	0.0571			0.0312	0.3071

Likelihood-ratio test of alpha=0: χ^2 (01) = 255.65 P ≥ χ^2 = 0.000.

Table 2. Random-intercept poisson regression model of the effect of time, larval developmental stage, imidacloprid dose, and rat feces collection day (Day) on the number of surviving *Lu. longipalpis* larvae feeding on lab rat feces. Statistical overdispersion is depicted by the parameter 'alpha'.

	Coef.	Std. Err	z	P	95% Conf. Interval	
Time	-0.0127	0.0063	-2.00	0.045	-0.0252	-0.0003
Stage	0.2913	0.0189	15.41	**0.000**	0.2543	0.3284
Day	-0.0823	0.0181	-4.54	0.000	-0.1179	-0.0468
Dose	-0.1250	0.0308	-4.05	0.000	-0.1855	-0.0645
Dose × time	-0.0231	0.0055	-4.18	0.000	-0.0339	-0.0122
Intercept	-0.1771	0.0571	-3.10	0.002	-0.2891	-0.0652
Linit	(offset)					
lnalpha	-4.4443	0.6089			-5.6378	-3.2509
alpha	0.0117	0.0071			0.0036	0.0387

Likelihood-ratio test of alpha=0: χ^2 (01) = 100.75 P ≥ χ^2 = 0.000.

Table 3. Random-intercept poisson regression model of the effect of time, larval developmental stage and imidacloprid dose, on the number of surviving *P. papatasi* larvae feeding on sand rat feces. Statistical overdispersion is depicted by the parameter 'alpha'.

	Coef.	Std. Err	z	*P*	*95% Conf. Interval*	
Time	-0.0374	0.0053	-6.95	0.000	-0.0479	-0.0268
Stage	0.3932	0.0182	21.59	0.000	0.3575	0.4289
Dose x time	-0.0019	0.0001	-30.01	0.000	-0.0020	-0.0017
Intercept	-0.2111	0.0347	-6.08	0.000	-0.2791	-0.1431
Linit	(offset)					
lnalpha	-4.1021	0.3664			-4.8202	-3.3840
alpha	0.0165	0.0061			0.0081	0.0339

Likelihood-ratio test of alpha=0: χ^2 (01) = 94.05, P ≥ χ^2 = 0.000.

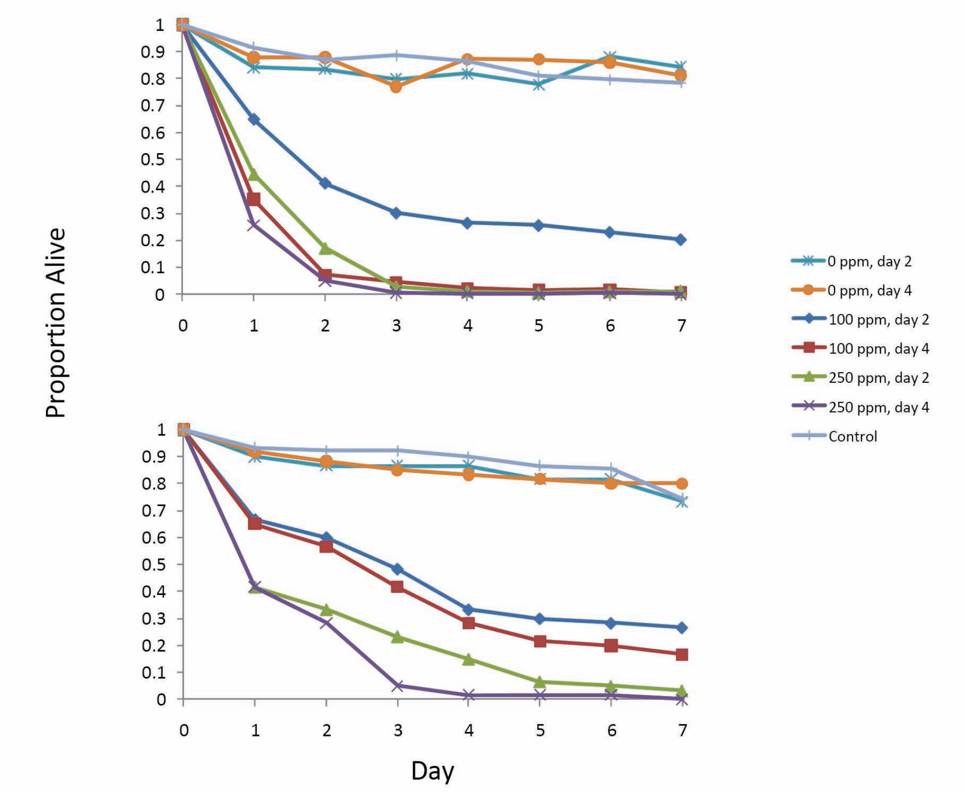

Figure 1. The effect of imidacloprid dose and rat feces collection day on the *P. papatasi* larvae survival during the seven days of the assay for 1st instar (top) and 2nd/3rd instar larvae (bottom).

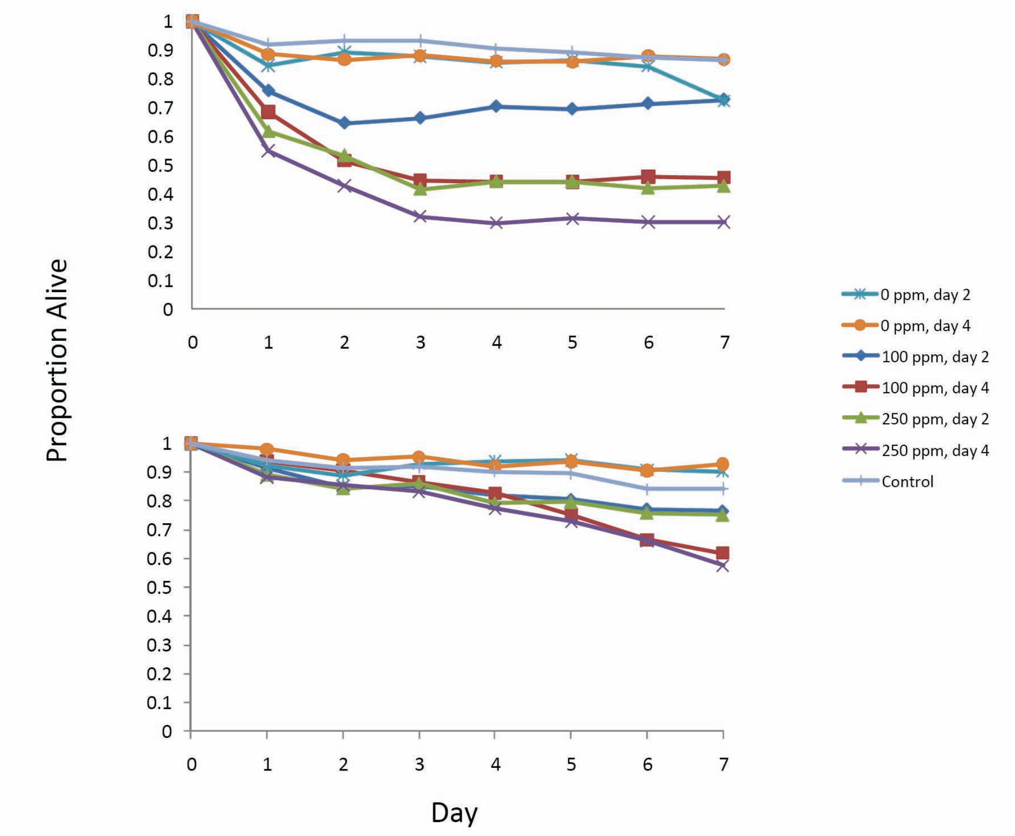

Figure 2. The effect of imidacloprid dose and rat feces collection day on the *L. longipalpis* larvae survival during the seven days of the assay for 1st instar (top) and 2nd/3rd instar larvae (bottom).

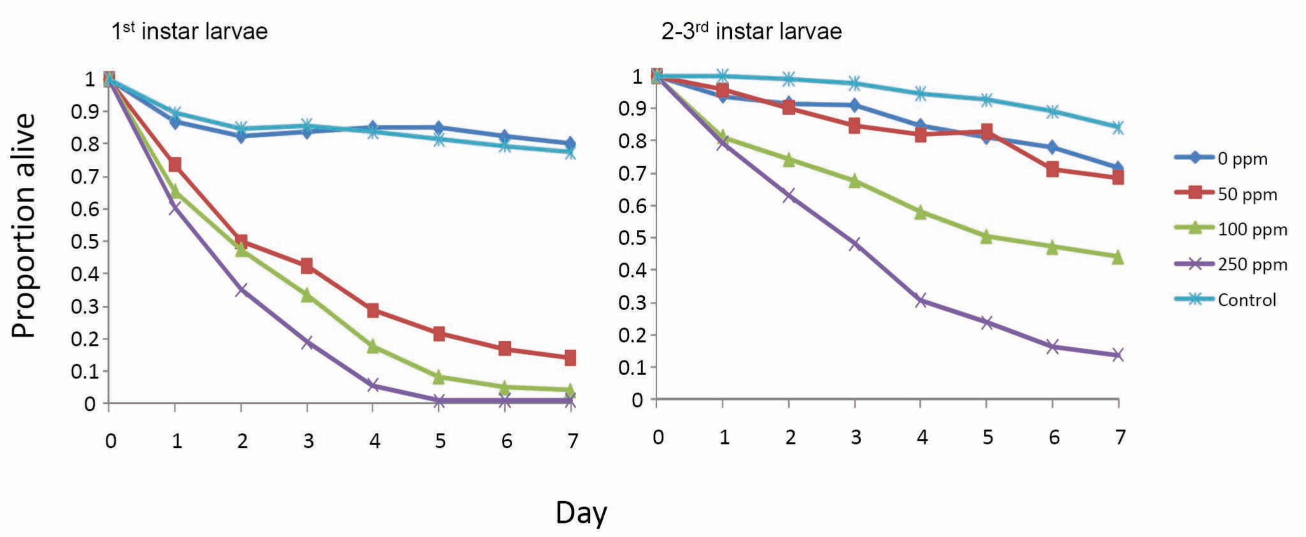

Figure 3. The effect of imidacloprid dose in sand rat diet on *P. papatasi* larvae survival during the seven days of the assay for 1st instar (top) and 2nd/3rd instar larvae (bottom).

Sand rat fecal pellets experiment

Similar to the rat fecal pellets experiments, survival of PPIS larvae decreased faster at higher doses of imidacloprid (Table 3, Figure 3). Calculated with respect to larval survival on day 7 (final day), the survival rate decreased at the average rate of 3% per unit dose (coef. = -0.0281, SE =0.0017, z=-16.69, P<0.0001). The survival rate of the later stages (2nd/3rd instar) was approximately 48% higher than that of 1st instar larvae (Table 3, Figure 3). For 1st intar larvae, insecticidal activity was demonstrated for all doses above 50 ppm, whereas for the 2nd/3rd instar this threshold was obtained only for the 250 ppm diet. Larval survival did not differ between the two control groups (coef. = -0.0430, se = 0.0329, z=-1.30, P= 0.193) (Figure 3).

Diet acceptance and short term physiological response of the rodents

Analyses of imidacloprid-treated rat pellets indicated that actual imidacloprid concentrations were 94.2 ± 0.8 ppm (100 ppm diet) and 246.2 ± 22.6 ppm (250 ppm diet). All laboratory rats accepted diets containing imidacloprid without any apparent health abnormalities, and all animals gained weight during the trials. Analyses of imidacloprid-treated sand rats indicated that actual imidacloprid concentrations were 49.0 ppm (50 ppm diet), 133.7 ppm (100 ppm diet), and 241.2 ppm (250 ppm diet). All sand rats accepted diets containing imidacloprid without any apparent health abnormalities. Sixteen sand rats gained weight during the trials and none lost more than 5 g (3% body weight), which was well within typical weekly weight variations. The mean quantity of feces per sand rat was 14.6 g (S.D. 1.4 g), with no significant difference between treatment groups (one-way ANOVA: P = 0.1199).

Effect of imidacloprid on survival of blood-feeding *P. papatasi* females

In a comparison of the two controls (i.e., alcohol-treated blood and untreated blood), we found that neither the proportion of surviving females (χ^2 =0.66, P=0.41) nor the proportion of blood-fed females (χ^2 =0.03, P=0.87) differed between the two groups (Table 4), which indicated that the alcohol solvent used for the insecticide treatments was not responsible for an increased mortality. In contrast, the proportion of surviving females feeding on either of the two insecticide concentrations was significantly lower compared with the control groups (χ^2 >79.12, P<0.0001). Similarly, the proportion of blood-fed females was significantly lower in the treatment groups compared with the control (χ^2 >33.81,

Table 4. The effect of imidacloprid-treated blood on the survival and number of blood-fed *P. papatasi* females.

	Controls		Imidacloprid-treated blood	
	Untreated blood	Alcohol-treated blood	1 mg/liter	5 mg/liter
Dead blood-fed	0	1	32	1
Dead unfed	0	1	29	43
Live blood-fed	51	42	2	0
Live unfed	2	2	12	5
Total	53	46	75	49
Proportion alive	1	0.96	0.19	0.10
Proportion blood-fed	0.96	0.93	0.45	0.02

P<0.0001) (Table 4). In comparing the two treatment groups, although the proportional survival at 5 mg/ml appeared lower than at 1 mg/ml, this difference was not significant (χ^2 =1.64, P=0.20). In contrast, the proportion of blood-fed sand flies was significantly lower at the higher dose (χ^2 =25.32, P < 0.0001) (Table 4).

DISCUSSION

The goal of this study was to provide proof-of-concept for the feasibility of applying systemic control for vector-host systems characterized by high degree of ecological coupling. Specifically, we evaluated the use of imidacloprid as a novel sand fly control agent. Our hypothesis was that by systemically affecting the reservoir host we could simultaneously affect the survival (adult female survival) and fecundity (larvae survival) of the sand fly.

The feed-through effect

The main portion of our study focused on determining the larvicidal feed-through effectiveness of imidacloprid in laboratory rat or sand rats. Whereas in previous studies researchers used a non-host rodent (hamster) model, a single sand fly species (*P. papatasi*), and a single larval stage (2nd instar) (Mascari et al. 2007a,b, Mascari et al. 2008), this study looked at a natural host and included another non-native species of sand fly. We used both a rodent model (laboratory rat) and the target reservoir host (fat sand rat), tested the effect of the feed-through effect on Old world and New world sand fly species (*P. papatasi and Lu. longipalpis*), and tested the effect of imidacloprid on two developmental stages (1st and 2nd/3rd instar larvae).

Our central prediction in this study was that larval survival rate would decrease faster with the increase in imidacloprid dose. This prediction was strongly supported in all three experiments. However, the 90% mortality rate criterion required for a substance to be considered larvicidal by the U.S. Environmental Protection Agency (Anonymous 1999) was not met in all cases. Using rat fecal pellets, we found that *Lu. longipalpis* was almost twice as resistant to imidacloprid as *P. papatasi* and did not pass the 90% threshold. This result reminds us that the effect of many insecticides is taxon-specific and its efficacy should be evaluated for each vector species prior to its application.

Larval developmental stage was found to be an important factor in determining the larvicidal effect of imidacloprid on larval survival. For 1st instar *P. papatasi* larvae feeding on rat pellets, larvicidal activity was observed at almost all dose levels, whereas for the older stages (2nd/3rd instar) larvicidal effect occurred only at 250 ppm. Similarly, for 1st instar *P. papatasi* larvae feeding on sand rat feces, larvicidal impact occurred at 100 and 250 ppm, whereas for 2nd/3rd instar larvae larvicidal effect was not obtained at either dose (although at 250 ppm 13.8% survival was observed) (Figure 3). This result stresses the importance of early season application of the treatment in order to impact larvae at their earlier and more vulnerable developmental stage.

The survival of *P. papatasi* larvae fed on rat feces was lower than for those fed on sand rat feces. This highlights an important point regarding the difference in using a model animal vs using the true reservoir animal. Rat feces are probably more nutritious for the larvae than those of sand rats, which due to their unique diet contain much more fiber material, resulting in slower feeding rate and potential longer absorption time of the insecticide. Thus, we strongly recommend the use of the true reservoir animal in similar studies.

The results also showed that larval survival was higher when fed on pellets collected at day 2 compared with day 4. This result indicates the importance of the bio-accumulation of the insecticide within the reservoir host tissues and calls for further studies regarding the length of time a diet should be applied to obtain an optimal accumulation of systemic dose. It also requires a study of the residual duration of the insecticide within the host tissues after cessation of the bait application. These points, together with the stage-specific differences of larval susceptibility to imidacloprid mentioned above, are critical for the field application of this method with respect to the timing and duration of the insecticide application.

Bait palatability and short-term health effects of treated rodents

A basic requirement of this method is that treated bait is acceptable by the rodent and that it does not have an adverse effect on the rodent's health. Based on short-term observations, our results suggest that for either lab rats or sand rats, imidacloprid does not have an adverse effect on health and does not compromise the palatability of the diet. Nevertheless, long-term confirmation of this is required and is in our future plans. It is encouraging that no rodent exhibited any health abnormalities, and all maintained or gained weight however, this issue warrants a long-term evaluation. Furthermore, it remains to be evaluated whether this formulation will be accepted by sand rats in the field. Sand rats are notorious for being very specific in their food preferences selecting only fresh chenepod vegetative parts (Daly and Daly 1973). In a preliminary trial in the Negev Desert, Israel (January, 2009), we tested a saltbush-based non-treated bait formulation that was found to be consumed by free-ranging sand rats. Subsequent field trials will be required to evaluate the rodents' preference for imidacloprid-treated bait.

Survival of blood-fed sand flies

As expected, the results confirmed that female sand flies feeding on imidacloprid-treated rabbit blood suffered a significant reduction in the their survival (Table 4). This result is consistent with previous studies that tested avermectins as a potential sand fly control agent (Kassem et al. 2001). In addition, we observed significant reduction in the proportion of blood-fed females in the insecticide treatments with the lowest proportion at the highest dose which also appeared to have the lowest survival. This result is apparently counter-intuitive because sand flies would have required a blood meal in order to be affected. The probable explanation is that the effect of the high dose was sufficiently lethal to affect the sand flies early during their foraging bout in which case we would not observe blood in the gut. However, this experiment only exhibited the potential adulticide effect of imidacloprid and the next stage would require feeding sand flies on rodents that were fed with imidacloprid-treated bait.

In summary, results of this study are encouraging with respect to the potential efficacy of imidacloprid as a systemic control agent for *P. papatasi* sand flies. Although some potential for affecting commensal non-target coprophagic invertebrates does exist, we expect that due to its local application near human settlements, this effect should be fairly minor. Hence, by taking advantage of the tight ecological association between the sand rat and the sand fly, (Ashford 1996, Wasserberg et al. 2002, Wasserberg et al. 2003), a potential exists for the development of a novel environment-friendly, sustainable, control system for cutaneous leishmaniasis (due to *L. major*). In such a system, rendering the reservoir host systemically affected with the insecticide would simultaneously affect the survival of both larval and adult stages. Field validation of this approach would be the next study stage. If successful, this system could be a model for other vector-host systems characterized by a tight ecological coupling of the vector and the rodent reservoir host, such as flea or tick-borne diseases.

Acknowledgments

This research was funded by a grant from the U.S. Department of Defense, Deployed War Fighter Protection Program, through the Armed Forces Pest Management Board. Studies were conducted while G. Wasserberg held a National Research Council (NRC) Research Associateship at the Walter Reed Army Institute of Research. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, MRC Publication, 1996 edition.

REFERENCES CITED

Alexander, B. and M. Maroli. 2003. Control of phlebotomine sandflies. Med. Vet. Entomol. 17: 1-18.

Anonymous. Product Performance, Test Guidelines. OPPTS 810.3700. Insect Repellents For Human Skin and Outdoor Premises. 1-250. 1999. Environmental Protection Agency.

Ashford, R.W. 1996. Leishmaniasis reservoirs & their significance in control. Clin. Dermatol. 14: 523-532.

Ashford, R.W. 1999. Cutaneous leishmaniasis: Strategies for prevention. Clin. Dermatol. 17: 327-332.

Ashford, R.W. 2000. The leishmaniases as emerging and reemerging zoonoses. Int. J. Parasitol. 30: 1269-1281.

Borchert, J.N., R.M. Davis, and R.M. Poché. 2009. Field efficacy of rodent bait containing the systemic insecticide imidacloprid against the fleas of California ground squirrels. J. Vector Ecol. 34: 92-98.

Borchert J.N. and R.M. Poché. 2003. Flea control on rodents using insect growth regulators and insecticides and estableeshing rodent and flea breeding colonies. Phase I technical report, 1-111. CDC SBIR Contract 200-2002-M-0511.

Borchert J.N. and R.M. Poché. 2004. A novel bait to reduce Lyme disease risk using systemic insecticides and insect growth regulators to control *Ixodes scapularis* Ticks on mice. Phase I technical report, 1-65. CDC SBIR Grant 2003-33610-13095.

Borchert J.N. and R.M. Poché. 2006. Novel pest control methods. 20060057178. U.S. patent application 20060057178.

Bruck, E., A. Elbert, R. Fischer, S. Krueger, J. Kuhnhold, A. M. Klueken, R. Nauen, J.F. Niebes, U. Reckmann, H.J. Schnorbach, R. Steffens, and X. van Waetermeulen. 2009. Movento (R), an innovative ambimobile insecticide for sucking insect pest control in agriculture: Biological profile and field performance. Crop Protect. 28: 838-844.

Daly, M. and S. Daly. 1973. On the feeding ecology of *Psammomys obesus* (Rodentia, Gerbillidae) in the Wadi Saoura, Algeria. Mammalia 37: 545-561.

Davis, R.M. 1999. Use of orally administered chitin inhibitor (Lufenuron) to control flea vectors of plague on ground squirrels in California. J. Med. Entomol. 36: 562-567.

Grafton-Cardwell, E.E., J.E. Lee, S.M. Robillard, and J.M. Gorden. 2008. Role of imidacloprid in integrated pest management of California citrus. J. Econ. Entomol. 101: 451-460.

Isman, M.B., O. Koul, J.T. Arnason, J. Stewart, and G.S. Salloum. 1991. Developing a neem-based insecticide for Canada. Mem. Entomol. Soc. Canada 159: 39-47.

Kassem, H.A., M.K. Tewfick, and B.M. El Sawaf. 2001. Evaluation of avermectins as sandfly control agents. Ann. Trop. Med. Parasitol. 95: 405-411.

Killick-Kendrick, R. 1999. The biology and control of phlebotomine sand flies. Clin. Dermatol. 17: 279-289.

Killick-Kendrick, R., M. Killick-Kendrick, C. Focheux, J. Dereure, M.P. Puech, and M.C. Cadiergues. 1997. Protection of dogs from bites of phlebotomine sandflies by deltamethrin collars for control of canine leishmaniasis. Med. Vet. Entomol. 11: 105-111.

Kloepper, J.W., C.M. Ryu, and S.A. Zhang. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology 94: 1259-1266.

Kuris, A.M., A.R. Blaustein, and J.J. Alio. 1980. Hosts as islands. Am. Nat. 116: 570-586.

Maroli, M., V. Mizzoni, C. Siragusa, A. D'Orazi, and L. Gradoni. 2001. Evidence for an impact on the incidence of canine leishmaniasis by the mass use of deltamethrin-impregnated dog collars in southern Italy. Med. Vet. Entomol. 15: 358-363.

Mascari, T.M., M.A. Mitchell, E.D. Rowton, and L.D. Foil. 2007a. Evaluation of novaluron as a feed-through insecticide for control of immature sand flies (Diptera : Psychodidae). J. Med. Entomol. 44: 714-717.

Mascari, T.M., M.A. Mitchell, E.D. Rowton, and L.D. Foil. 2007b. Laboratory evaluation of diflubenzuron as a feed-through for control of immature sand flies (Diptera : Psychodidae). J. Med. Entomol. 44: 171-174.

Mascari, T.M., M.A. Mitchell, E.D. Rowton, and L.D. Foil. 2008. Ivermectin as a rodent feed-through insecticide for control of immature sand flies (Diptera : Psychodidae). J. Am. Mosq. Contr. Assoc. 24: 323-326.

Metzger, M.E. and M.K. Rust. 2002. Laboratory evaluation of fipronil and imidacloprid topical insecticides for control of the plague vector *Oropsylla montana* (Siphonaptera : Ceratophyllidae) on California ground squirrels (Rodentia : Sciuridae). J. Med. Entomol. 39: 152-161.

Mullen G. and L. Durden. 2009. *Medical and Veterinary Entomology*. 2nd edition. Academic Press, London, UK.

Mullins, J.W. 1993. Imidacloprid - a new nitroguanidine insecticide. Am. Chem. Soc. Symp. Ser. 524: 183-198.

Rabe-Hesketh S. and A. Skrondal. 2008. *Multilevel and Longitudinal Modeling Using Stata*., 2nd edition. Stata Press.

Reithinger, R., P.G. Coleman, B. Alexander, E.P. Vieira, G. Assis, and C.R. Davies. 2004. Are insecticide-impregnated dog collars a feasible alternative to dog culling as a strategy for controlling canine visceral leishmaniasis in Brazil? Int. J. Parasitol. 34: 55-62.

Saliba, E.K. and O.Y. Oumeish. 1999. Reservoir hosts of cutaneous leishmaniasis. Clin. Dermatol. 17: 275-277.

Service, M. 2008. *Medical Entomology for Students*. 4th edition. Cambridge University Press, Cambridge, UK.

Slowik, T.J., R.S. Lane, and R.M. Davis. 2001. Field trial of systemically delivered arthropod development-inhibitor (Fluazuron) used to control woodrat fleas (Siphonaptera : Ceratophyllidae) and ticks (Acari : Ixodidae). J. Med. Entomol. 38: 75-84.

StataCorp. 2007. Stata Statistical software. Release 10 edition.

Tesh, R.B. and G.B. Modi. 1984. A simple method for experimental-infection of phlebotomine sand flies with *Leishmania*. Am. J. Trop. Med. Hyg. 33: 41-46.

Wasserberg, G., Z. Abramsky, G. Anders, M. El Fari, G. Schoenian, L. Schnur, B.P. Kotler, I. Kabalo, and A. Warburg. 2002. The ecology of cutaneous leishmaniasis in Nizzana, Israel: infection patterns in the reservoir host, and epidemiological implications. Int. J. Parasitol. 32: 133-143.

Wasserberg, G., Z. Abramsky, B.P. Kotler, R.S. Ostfeld, I. Yarom, and A. Warburg. 2003. Anthropogenic disturbances enhance occurrence of cutaneous leishmaniasis in Israel deserts: patterns and mechanisms. Ecol. Appl. 13: 868-881.

Weintraub, P.G. 2007. Insect vectors of phytoplasmas and their control - an update. Bull. Insectol. 60: 169-173.

Young, D.G., P.V. Perkins, and R.G. Endris. 1981. A larval diet for rearing Phlebotomine sand flies (Diptera, Psychodidae). J. Med. Entomol. 18: 446.